

US 20030157552

BEST AVAILABLE COPY

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
30 August 2001 (30.08.2001)

PCT

(10) International Publication Number  
WO 01/62954 A2

(51) International Patent Classification: C12Q 1/00

(21) International Application Number: PCT/US01/05855

(22) International Filing Date: 23 February 2001 (23.02.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/184,526 24 February 2000 (24.02.2000) US  
60/221,697 31 July 2000 (31.07.2000) US  
60/255,771 15 December 2000 (15.12.2000) US

(71) Applicants: XENON GENETICS, INC. [CA/CA];  
520 West Sixth Avenue, Suite 510, Vancouver, British  
Columbia V5Z 4H5 (CA). WISCONSIN ALUMNI RE-  
SEARCH FOUNDATION [US/US]; 614 Walnut Street,

13th Floor, Madison, WI 53705 (US). UNIVERSITY OF  
BRITISH COLUMBIA University Industry Liaison  
Office [CA/CA]; 2075 Westbrook Mall, Vancouver, British  
Columbia V6T 1W5 (CA).

(72) Inventors: BROWNLIE, Alison, J.; 5320 West Broad-  
way, Apartment 101, Vancouver, British Columbia V6R  
2BC (CA). HAYDEN, Michael, R.; 4484 West 7th  
Avenue, Vancouver, British Columbia V6R 1W9 (CA).  
ATTIE, Alan, D.; 1906 Vilas Avenue, Madison, WI  
53711 (US). NTAMBI, James, M.; 4730 Waukesha  
Street, Madison, WI 53705 (US). GRAY-KELLER,  
Mark, P.; 4558 Stonewood Drive, Middleton, WI 53562  
(US). MIYAZAKI, Makoto; 4801 Sheboygan Avenue,  
Madison, WI (US).

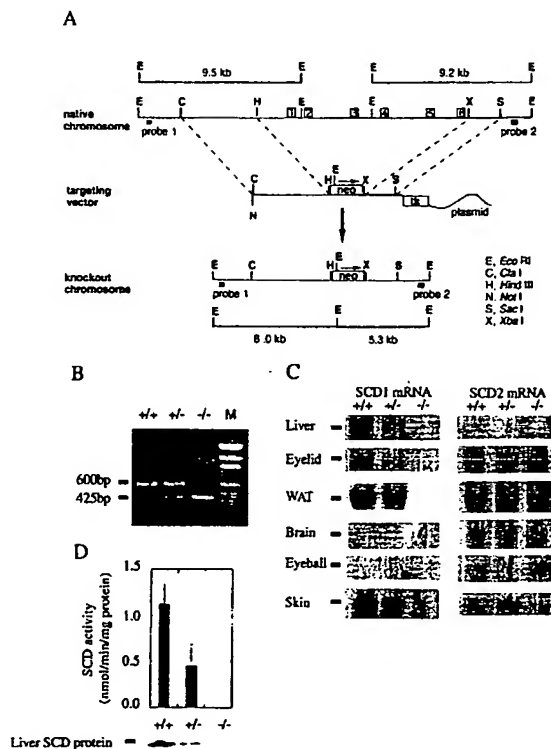
(74) Agents: GRANT, Alan, J. et al.; Carella, Byrne, Bain,  
Gilfillan, Cecchi, Stewart & Olstein, 6 Becker Farm Road,  
Roseland, NJ 07068 (US).

[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS USING STEAROYL-CoA DESATURASE TO IDENTIFY TRIGLYCERIDE REDUCING THERAPEUTIC AGENTS



WO 01/62954 A2



(57) Abstract: The use of screening assays based on the role of human stearoyl-CoA desaturase-1 ("hSCD1") in human diseases, disorders or conditions relating to serum levels of triglyceride, VLDL, HDL, LDL, total cholesterol, or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like, is disclosed. Also disclosed are conventions useful in the prevention and/or treatment of such diseases.

object of this invention to provide compositions for use in treating these disease, disorders and related conditions.

5

## BRIEF SUMMARY OF THE INVENTION

This invention discloses, for the first time, the role of human stearoyl-CoA desaturase-1 ("hSCD1") in a wide range of human diseases and disorders. In particular, SCD1 biological activity in humans is directly related  
10 to serum levels of triglycerides and VLDL. In addition, SCD1 biological activity also affects serum levels of HDL, LDL, and/or total cholesterol, reverse cholesterol transport, and the production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and/or the like.

15 It is an object of the present invention to provide a process or screening assay for identifying, from a library of test compounds, a therapeutic agent which modulates the biological activity of said human stearoyl-CoA desaturase (hSCD1) and is useful in treating a human disorder or condition relating to serum levels of triglyceride or VLDL. Preferably, the  
20 screening assay identifies inhibitors of hSCD1 which lower serum triglyceride levels and provide an important cardioprotective benefit for humans.

It is also an object of the present invention to provide a process or screening assay for identifying, from a library of test compounds, a  
25 therapeutic agent which modulates the biological activity of said human stearoyl-CoA desaturase (hSCD1) and is useful in treating a human disorder or condition relating to serum levels of HDL, LDL, and/or total cholesterol, reverse cholesterol transport or the production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and/or the like

30

In one aspect, the present invention relates to vectors comprising human stearoyl-CoA desaturase (hSCD1) genes and promoter sequences

and to recombinant eukaryotic cells, and cell lines, preferably mammalian cells, and most preferably human cells, and cell lines, transfected so as to comprise such vectors and/or said polynucleotides and wherein said cells express hSCD1. Disclosed herein is the full length promoter sequence for  
5 hSCD1, SEQ ID. No. 1.

It is also an object of the present invention to provide agents capable of modulating the activity and/or expression of human stearyl-CoA desaturase 1 (hSCD1) as disclosed herein, especially where said modulating ability was  
10 first determined using an assay comprising hSCD1 biological activity or a gene encoding hSCD1. Pharmaceutical compositions comprising such agents are specifically contemplated.

It is a still further object of the present invention to provide agents  
15 wherein said agent is useful in treating, preventing and/or diagnosing a disease or condition relating to hSCD1 biological activity.

It is a yet further object of the present invention to provide a process for preventing or treating a disease or condition in a patient afflicted therewith  
20 comprising administering to said patient a therapeutically or prophylactically effective amount of a composition as disclosed herein.

In a pharmacogenomic application of this invention, an assay is provided for identifying cSNPs (coding region single nucleotide  
25 polymorphisms) in hSCD1 of an individual which are associated with human disease processes or response to medication.

In other aspects, the present invention also provides a process for diagnosing a disease or condition in a patient, commonly a human being,  
30 suspected of being afflicted therewith, or at risk of becoming afflicted therewith, comprising obtaining a tissue sample from said patient and determining the level of activity of hSCD1 in the cells of said tissue sample

and comparing said activity to that of an equal amount of the corresponding tissue from a patient not suspected of being afflicted with, or at risk of becoming afflicted with, said disease or condition.

5           In other aspects, the present invention also provides a process for diagnosing a disease or condition in a patient, commonly a human being, suspected of being afflicted therewith, or at risk of becoming afflicted therewith, comprising obtaining a tissue sample from said patient and identifying mutations in the hSCD1 gene in the cells of said tissue sample  
10       and comparing said gene to that of a corresponding tissue from a patient not suspected of being afflicted with, or at risk of becoming afflicted with, said disease or condition.

15

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Generation of SCD1 null mice (A) Targeting strategy for SCD1. A partial map of the genomic locus surrounding the Scd1 locus is  
20       shown. Homologous recombination resulted in the replacement of exons 1-6 by neo 7 gene. Gene-targeting events were verified by Southern blot analysis using EcoRI and probe A or B or by PCR analysis. (B) PCR analysis demonstrating SCD <sup>-/-</sup> mice. In breeding heterozygotes, wild-type, heterozygotes and homozygotes were born in Mendelian fashion (+/+; +/-; -/-  
25       = 21: 43: 20  $\chi^2=0.395$ ). (C) Northern blot analysis. 20 $\mu$ g of total RNA was isolated from the liver and subjected to Northern blot analyses. Blots were probed with a mouse SCD1 and 2 cDNA fragments. (D) Immunoblot analysis of liver showed the absence of immunoreactive SCD in SCD1 <sup>-/-</sup> mice, whereas SCD1 protein was detected in liver tissue from both wild-type and  
30       heterozygote mice in a manner dependent on gene dosage. (E) Liver SCD activity was abolished in SCD <sup>-/-</sup> mice. As mentioned above, heterozygotes present an intermediate phenotype when compared to wild-type and null

WHAT IS CLAIMED IS:

1. A method for identifying, from a library of test compounds, a  
therapeutic agent which is useful in humans for the treatment of a  
disorder or condition relating to serum levels of triglyceride or VLDL  
comprising
  - a) providing a screening assay having SCD1 biological activity;
  - b) contacting said screening assay with a test compound; and
  - c) subsequently measuring said biological activity;wherein a test compound which modulates said biological activity is  
said therapeutic agent, or an analog thereof.
2. A screening assay for identifying compounds useful in human for the  
treatment of a disorder or condition relating to serum levels of  
triglyceride, or VLDL, said screening assay comprising
  - a) a screening assay having SCD1 biological activity;wherein a test compound which modulates said SCD1 biological  
activity in said screening assay is a compound, or an analog  
thereof, which is useful for said treatment.
3. A method for identifying, from a library of test compounds, a  
therapeutic agent which is useful in humans for the treatment of a  
disorder or condition relating to serum levels of triglyceride or VLDL,  
comprising
  - a) an assay having measurable SCD1 biological activity;wherein a test compound that modulates SCD1 biological activity upon  
contact with said assay is said therapeutic agent or an analog thereof.
4. The assay of claim 1-3 wherein said compound is an antagonist or  
inhibitor of SCD1 biological activity.

5. The assay of claim 1-3 wherein said compound is an agonist of SCD1 biological activity
6. The assay of claims 1-5 wherein inhibitor does not substantially inhibit  
5 the biological activity in a human of a delta-5 desaturase, delta-6 desaturase or fatty acid synthetase
7. The assay of claims 1-5 further comprising the step of assaying said  
10 therapeutic agent to further select compounds which do not substantially inhibit in a human the activity of delta-5 desaturase, delta-6 desaturase or fatty acid synthetase.
8. The screening assay of claim 1-3, wherein SCD1 biological activity is  
15 measured by an assay selected from among:
  - a) SCD1 polypeptide binding affinity;
  - b) SCD1 desaturase activity in microsomes;
  - c) SCD1 desaturase activity in a whole cell assay
  - d) quantification of SCD1 gene expression level; and
  - e) quantification of SCD1 protein level.  
20
9. A cell line containing a recombinant SCD1 protein.
10. A cell line containing the recombinant SCD1 protein of claim 9 which is  
25 used in a screening assay for identifying compounds that inhibit SCD1 biological activity and are useful for treatment in a human of a disorder or condition relating to serum levels of triglyceride or VLDL
11. An assay employing the cell line of claim 9 wherein the identified  
30 compound is further selected from among those compounds that do not substantially inhibit in humans the biological activity of delta-5 desaturase, delta-6 desaturase or fatty acid synthetase.

12. A recombinant cell line comprising the SCD1 promoter nucleic acid sequence of SEQ ID No. 1 operably linked to a reporter gene construct.
- 5
13. Use of the recombinant cell line of claim 12 in a screening assay for identifying compounds which are useful for the treatment in humans of a disorder or condition relating to serum levels of triglyceride or VLDL.
- 10
14. An isolated stearyl-CoA desaturase (SCD) nucleic acid encoded by the polynucleotide sequence comprising SEQ ID No. [SCD1 cDNA]
15. A reporter gene construct comprising the SCD1 promoter nucleic acid sequence of SEQ ID No. 1 operably linked to a reporter gene.
- 15
16. A vector comprising the nucleic acid of claim 14 or 15.
17. An isolated stearyl-CoA desaturase protein.
- 20
18. A method for identifying a compound which binds to or interacts with the polypeptide of claim 17 comprising:
- a) contacting the polypeptide of claim 17 or a cell expressing the polypeptide of claim 17 with a test compound; and
- b) determining whether the polypeptide binds to or interacts with the test compound.
- 25
19. The method of claim 18 wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:
- 30
- a) detection of binding by direct detection of test compound/polypeptide binding;
- b) detection of binding using a competition binding assay; and

c) detection of binding using an assay for SCD1 biological activity.

20. A method for modulating the activity of the polypeptide of claim 17 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in sufficient amount to modulate the activity of the polypeptide.
21. A screening assay employing SCD1 nucleic acid of claim 14 and/or SCD1 polypeptide of claim 17 for use in identifying compounds useful for treatment of a disorder or condition relating to serum levels of triglyceride or VLDL.
22. A method of treating a disease or condition in a human selected from among a disorder or condition relating to serum levels of triglyceride or VLDL, said method consisting essentially of inhibition of the activity of SCD1 protein in said human.
23. The method of claim 22, wherein said inhibitor does not substantially inhibit activity of delta-5 desaturase, delta-6 desaturase or fatty acid synthetase.
24. Use of a compound for treatment of a disorder or condition relating to serum levels of triglyceride or VLDL, wherein said compound or analog thereof was identified by its ability to modulate SCD1 biological activity in an assay of claim 1-3.
25. Use of a compound in a human for treatment of a disorder or condition relating to serum levels of triglyceride or VLDL, wherein said use of said compound or analog thereof was first identified by said compound's ability to modulate SCD1 biological activity in an assay of claim 1-3.



26. A modulator of SCD1 biological activity which is useful in humans for treatment of a disorder or condition relating to serum levels of triglyceride or VLDL, identified by a screening assay wherein said modulator detectably modulates SCD1 biological activity.
- 5 27. A composition which is useful in humans for treatment of a disorder or condition relating to serum levels of triglyceride or VLDL, first identified by a screening assay wherein said composition modulates the biological activity of SCD1.
- 10 28. A method for identifying, from a library of test compounds, a therapeutic agent which is useful in humans for the treatment of a disorder or condition relating to serum levels of triglyceride, VLDL, HDL, LDL, total cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like, comprising
- 15 a) providing a screening assay having a measurable biological activity of a vertebrate delta-9 stearoyl-CoA desaturase;
- b) contacting said screening assay with a test compound; and
- c) subsequently measuring said biological activity;
- 20 wherein a test compound which modulates said biological activity is said therapeutic agent, or an analog thereof.
29. Use of a compound in humans for treatment of a disorder or condition relating to serum levels of triglyceride, VLDL, HDL, LDL, total
- 25 cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like, wherein said compound or analog thereof was identified by its ability to modulate a measurable biological activity of a vertebrate delta-9 stearoyl-CoA desaturase in an assay of claim 28.
- 30 30. Use of a compound in humans for treatment of a disorder or condition relating to serum levels of triglyceride, VLDL, HDL, LDL, total

- cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like, wherein said use of said compound or analog thereof was first identified by said compound's ability to modulate a measurable biological activity of a vertebrate delta-9 stearoyl-CoA desaturase in an assay of claim 28.
- 5
31. The method of claims 28 - 30 wherein the compound does not in humans substantially inhibit fatty acid synthetase, delta-5 desaturase or delta-6 desaturase.
- 10 32. A method for up-regulating the ABCA1 gene in an individual comprising the step of administering to that individual an agent which lowers the level of activity of stearoyl-CoA desaturase (SCD1) protein in that individual.
- 15 33. A method as claimed in claim 32 wherein the agent is an inhibitor which inhibits the enzymatic activity of the SCD1 protein.
34. A method as claimed in claim 33 wherein the inhibitor is selected from the group consisting of a thia-fatty acid, a conjugated linoleic acid, and a cyclopropenoid fatty acid.
- 20 35. A method as claimed in claim 34 wherein the thia-fatty acid is selected from the group consisting of a 9-thiastearic acid and a fatty acid having a sulfoxy moiety.
36. A method as claimed in claim 34 wherein the conjugated linoleic acid is a trans-10, cis 12 conjugated linoleic acid.
- 25 37. A method as claimed in claim 34 wherein the cyclopropenoid fatty acid is selected from the group consisting of steric acid and malvalic acid.
38. A method as claimed in claim 32 wherein the agent inhibits the SCD1 protein by inhibiting the transcription of an SCD1 gene.

39. A method as claimed in claim 38 wherein the inhibitor is selected from the group consisting of a thiazoladinedione compound and a polyunsaturated fatty acid.
- 5 40. A method as claimed in claim 39 wherein the thiazoladinedione compound is selected from the group consisting of BRL49653, Pioglitazone, Ciglitazone, Englitazone, and Troglitazone.
41. A method as claimed in claim 39 wherein the polyunsaturated fatty acid is selected from the group consisting of dodecahexaenoic acid, arachidonic acid, and linoleic acid.
- 10 42. A method as claimed in claim 33 wherein the inhibitor inhibits the SCD1 protein by inhibiting a protein selected from the group consisting of a cytochrome b<sub>5</sub> protein, a NADH-cytochrome b<sub>5</sub> reductase protein, and a terminal cyanide-sensitive desaturase protein.
- 15 43. A method for elevating high density lipoprotein (HDL) particles in an individual comprising the step of administering to that individual an inhibitor of an SCD1 protein.
44. A method for reducing very low density lipoprotein (VLDL) particles in an individual comprising the step of administering to that individual an inhibitor of an SCD1 protein.
- 20 45. A method for reducing plasma triglycerides particles in an individual comprising the step of administering to that individual an inhibitor of an SCD1 protein.
- 25 46. A method for enhancing the cellular efflux of phospholipids and/or cholesterol in an individual comprising the step of administering to that individual an inhibitor of an SCD1 protein.
47. A method for inhibiting atherosclerosis in an individual comprising the step of administering to that individual an inhibitor of an SCD1 protein.

48. A method for treating diabetes and insulin resistance in an individual comprising the step of administering to that individual an inhibitor of an SCD1 protein expression or activity.
- 5 49. A method of testing compounds for their effects on ABCA1 activity comprising the steps of feeding an amount of the test compounds to a subject and monitoring the effect on the level of SCD1 activity in the subject.
- 10 50. A method for identifying, from a library of test compounds, a therapeutic agent which is useful in humans for the treatment of a disorder or condition relating to serum levels of HDL, LDL, total cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like, comprising
- 15 a) providing a screening assay having SCD1 biological activity;  
b) contacting said screening assay with a test compound; and  
c) subsequently measuring said biological activity;  
wherein a test compound which modulates said biological activity is said therapeutic agent, or an analog thereof.
- 20 51. A screening assay for identifying compounds useful in human for the treatment of a disorder or condition relating to serum levels of HDL, LDL, total cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like, said screening assay comprising
- 25 a) a screening assay having SCD1 biological activity;  
wherein a test compound which modulates said SCD1 biological activity in said screening assay is a compound, or an analog thereof, which is useful for said treatment.
- 30 52. A method for identifying, from a library of test compounds, a therapeutic agent which is useful in humans for the treatment of a

- disorder or condition relating to serum levels of HDL, LDL, total cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like, comprising
- 5 a) an assay having measurable SCD1 biological activity;  
wherein a test compound that modulates SCD1 biological activity upon contact with said assay is said therapeutic agent or an analog thereof.
- 10 53. The assay of claim 1-3 wherein said compound is an antagonist or inhibitor of SCD1 biological activity.
54. The assay of claim 1-3 wherein said compound is an agonist of SCD1 biological activity
- 15 55. The assay of claims 1-5 wherein inhibitor does not substantially inhibit the biological activity in a human of a delta-5 desaturase, delta-6 desaturase or fatty acid synthetase
- 20 56. The assay of claims 1-5 further comprising the step of assaying said therapeutic agent to further select compounds which do not substantially inhibit in a human the activity of delta-5 desaturase, delta-6 desaturase or fatty acid synthetase.
- 25 57. The screening assay of claim 1-3, wherein SCD1 biological activity is measured by an assay selected from among:  
a) SCD1 polypeptide binding affinity;  
b) SCD1 desaturase activity in microsomes;  
c) SCD1 desaturase activity in a whole cell assay  
d) quantification of SCD1 gene expression level; and  
30 e) quantification of SCD1 protein level.
58. A cell line containing a recombinant SCD1 protein.

59. A cell line containing the recombinant SCD1 protein of claim 9 which is used in a screening assay for identifying compounds that inhibit SCD1 biological activity and are useful for treatment in a human of a disorder or condition relating to serum levels of HDL, LDL, total cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like.
60. An assay employing the cell line of claim 9 wherein the identified compound is further selected from among those compounds that do not substantially inhibit in humans the biological activity of delta-5 desaturase, delta-6 desaturase or fatty acid synthetase.
61. A recombinant cell line comprising the SCD1 promoter nucleic acid sequence of SEQ ID No. 1 operably linked to a reporter gene construct.
62. Use of the recombinant cell line of claim 12 in a screening assay for identifying compounds which are useful for the treatment in humans of a disorder or condition relating to serum levels of HDL, LDL, total cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like.
63. An isolated stearoyl-CoA desaturase nucleic acid encoded by the polynucleotide sequence comprising SEQ ID NO. 1.
64. A reporter gene construct comprising the SCD1 promoter nucleic acid sequence of SEQ ID No. 1 operably linked to a reporter gene.
65. A vector comprising the nucleic acid of claim 14 or 15.

66. An isolated stearyl-CoA desaturase protein.
67. A method for identifying a compound which binds to or interacts with the polypeptide of claim 17 comprising:
- 5 a) contacting the polypeptide of claim 17 or a cell expressing the polypeptide of claim 17 with a test compound; and
- b) determining whether the polypeptide binds to or interacts with the test compound.
- 10 68. The method of claim 18 wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:
- a) detection of binding by direct detection of test compound/polypeptide binding;
- 15 b) detection of binding using a competition binding assay; and
- c) detection of binding using an assay for SCD1 biological activity.
69. A method for modulating the activity of the polypeptide of claim 17 comprising contacting the polypeptide or a cell expressing the
- 20 polypeptide with a compound which binds to the polypeptide in sufficient amount to modulate the activity of the polypeptide.
70. A screening assay employing SCD1 nucleic acid of claim 14 and/or SCD1 polypeptide of claim 17 for use in identifying compounds useful for treatment of a disorder or condition relating to serum levels of HDL,
- 25 LDL, total cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like.
71. A method of treating a disease or condition in a human selected from among a disorder or condition relating to serum levels of HDL, LDL,
- 30 total cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids,

wax esters, and the like, said method consisting essentially of inhibition of the activity of SCD1 protein in said human.

- 5        72. The method of claim 22, wherein said inhibitor does not substantially inhibit activity of delta-5 desaturase, delta-6 desaturase or fatty acid synthetase.
- 10       73. Use of a compound for treatment of a disorder or condition relating to serum levels of HDL, LDL, total cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like, wherein said compound or analog thereof was identified by its ability to modulate SCD1 biological activity in an assay of claim 1-3.
- 15       74. Use of a compound in a human for treatment of a disorder or condition relating to serum levels of HDL, LDL, total cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like, wherein said use of said compound or analog thereof was first identified by said compound's ability to modulate SCD1 biological activity in an assay of claim 1-3.
- 20       75. A modulator of SCD1 biological activity which is useful in humans for treatment of a disorder or condition relating to serum levels of HDL, LDL, total cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like, identified by a screening assay wherein said modulator detectably modulates SCD1 biological activity.
- 25       76. A composition which is useful in humans for treatment of a disorder or condition relating to serum levels of HDL, LDL, total cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like,



first identified by a screening assay wherein said composition modulates the biological activity of SCD1.

- 5           77. A process for identifying a SCD1-modulating agent, comprising:  
a) contacting under physiological conditions a chemical agent and a molecule having or inducing SCD1 activity;  
b) detecting a change in the activity of said molecule having or inducing SCD1 activity following said contacting;  
thereby identifying an SCD1 modulating agent.
- 10       78. The process of claim 77 wherein said molecule having or inducing SCD1 activity is a polypeptide having such activity.
- 15       79. The process of claim 77 wherein said molecule having or inducing SCD1 activity is a polynucleotide encoding a polypeptide having such activity.
- 20       80. The process of claim 77 wherein said molecule having or inducing SCD1 activity is a polypeptide modulating the activity of a polynucleotide encoding a polypeptide having such activity.
- 25       81. The process of claim 77 wherein said change in activity is an increase in activity.
82. The process of claim 77 wherein said change in activity is a decrease in activity.
- 30       83. The process of claim 77 wherein said contacting is accomplished *in vivo*.
84. The process of claim 83 wherein said contacting in step (a) is accomplished by administering said chemical agent to an animal afflicted with a triglyceride (TG)- or very low density lipoprotein

(VLDL)-related disorder and subsequently detecting a change in plasma triglyceride level in said animal thereby identifying a therapeutic agent useful in treating a triglyceride (TG)- or very low density lipoprotein (VLDL)-related disorder.

5

85. The process of claim 84 wherein said animal is a human.

86. The process of claim 84 wherein said change in SCD1 activity in said animal is a decrease in activity.

10

87. The process of claim 84 or 85 wherein said SCD1 modulating agent does not substantially inhibit the biological activity of a delta-5 desaturase, delta-6 desaturase or fatty acid synthetase.

15 88. The process of claim 77 or 84 wherein said detectable change in SCD1 activity is detected by detecting a change in:

- a) SCD1 polypeptide binding affinity;
- b) SCD1 desaturase activity in microsomes;
- c) SCD1 desaturase activity in a whole cell;
- 20 d) SCD1 gene expression; or
- e) SCD1 protein level.

89 A recombinant cell line comprising a recombinant SCD1 protein.

25 90. The process of claim 88 wherein said whole cell of (c) is derived from the cell line of claim 89.

91. The process of claim 90 wherein said SCD1 modulating agent does not substantially inhibit in humans the biological activity of delta-5 desaturase, delta-6 desaturase or fatty acid synthetase.

30

92. A recombinant cell line comprising the SCD1 promoter nucleic acid sequence of SEQ ID No. 1 operably linked to a reporter gene construct.
- 5 93. The process of claim 88 wherein said whole cell of (c) is derived from the cell line of claim 92.
94. An isolated stearyl-CoA desaturase encoded by the polynucleotide sequence comprising SEQ ID No. 1.
- 10 95. A reporter gene construct comprising the SCD1 promoter nucleic acid sequence of SEQ ID No. 1 operably linked to a reporter gene.
96. A vector comprising the nucleic acid of claim 94 or 95.
- 15 97. An isolated polypeptide having stearyl-CoA reductase activity.
98. A process for identifying a chemical agent that binds to or interacts with the polypeptide of claim 97 comprising:
- 20 a) contacting the polypeptide of claim 97 or a cell expressing the polypeptide of claim 97 with a chemical agent; and
- b) detecting binding or interaction of the chemical agent with said polypeptide.
99. The process of claim 98 wherein the binding of the chemical agent to the polypeptide is detected by a method selected from the group consisting of:
- 25 a) direct detection of chemical agent/polypeptide binding;
- b) detection of binding by competition binding assay; and
- c) detection of binding by assay for SCD1 biological activity.
- 30 100. A process for modulating the activity of the polypeptide of claim 97 comprising contacting the polypeptide or a cell expressing the

polypeptide with a compound that binds to the polypeptide in sufficient amount to modulate the activity of the polypeptide.

- 5 102. The process of claim 77 or 84 wherein said molecule having or inducing SCD1 activity is selected from the group consisting of the SCD1 nucleic acid of claim 94 and/or SCD1 polypeptide of claim 97.
103. A process for treating a human afflicted with a disease or condition relating to serum levels of triglyceride or VLDL comprising inhibiting SCD1 activity in said human.
- 10 104. The method of claim 100 wherein said inhibition of SCD1 activity is not accompanied by substantial inhibition of activity of delta-5 desaturase, delta-6 desaturase or fatty acid synthetase.
- 15 105. A process for treating a human patient afflicted with a disorder or condition relating to serum levels of triglyceride or VLDL comprising administering to said patient a therapeutically effective amount of an agent whose therapeutic activity was first identified by the process of claim 77 or 84.
- 20 106. A modulator of SCD1 activity which is useful in humans for treatment of a disorder or condition relating to serum levels of triglyceride or VLDL wherein said activity was first identified by its ability to modulate SCD1 activity.
- 25 107. A process for identifying a vertebrate delta-9 stearyl-CoA desaturase-modulating agent, comprising:
- a) contacting under physiological conditions a chemical agent and a molecule having or inducing vertebrate delta-9 stearyl-CoA desaturase activity;
  - b) detecting a change in the activity of said molecule having or inducing vertebrate delta-9 stearyl-CoA desaturase activity following said contacting;

thereby identifying a vertebrate delta-9 stearyl-CoA desaturase modulating agent.

- 5 109. The process of claim 106 wherein said contacting in step (a) is accomplished by administering said chemical agent to an animal afflicted with a disorder or condition related to serum levels of triglyceride, VLDL, HDL, LDL, total cholesterol, reverse cholesterol transport or production or secretion of mucous membranes, monounsaturated fatty acids, wax esters, and like parameters,
- 10 detecting a change in the activity of said molecule having or inducing vertebrate delta-9 stearyl-CoA desaturase activity following said contacting and thereby identifying a therapeutic agent useful in treating a triglyceride, VLDL, HDL, LDL, total cholesterol, or production or secretion of mucous membranes, monounsaturated fatty acids, wax
- 15 esters, and like disease-related disorder.
109. A process for treating a human patient afflicted with a disease or condition relating to serum levels of triglyceride, VLDL, HDL, LDL, total cholesterol, reverse cholesterol transport or production or secretion of
- 20 mucous membranes, monounsaturated fatty acids, wax esters, and like parameters, comprising administering to said human patient a therapeutically effective amount of an agent for which such therapeutic activity was identified by the process of claim 106.
- 25 110. The process of claim 107-109 wherein the modulating agent does not substantially inhibit fatty acid synthetase, delta-5 desaturase or delta-6 desaturase of humans.
111. A process for identifying, from a library of test compounds, a therapeutic agent which is useful in humans for the treatment of a disorder or condition relating to serum levels of triglyceride or very low
- 30 density lipoprotein (VLDL) comprising

- a) providing a screening assay having stearyl-Coenzyme A desaturase type 1 (SCD1) biological activity as a component thereof;
- b) contacting said SCD1 activity with a test compound;
- 5 c) administering to a human a compound found to modulate said activity in (b); and
- (d) detecting a change in serum level of triglyceride or VLDL in said human following said administering;
- 10 thereby identifying an agent useful in the treatment of a disorder or condition relating to serum levels of triglyceride or very low density lipoprotein (VLDL) .
112. The process of claim 111 wherein said agent is an antagonist or inhibitor of SCD1 biological activity.
- 15 113. The process of claim 111 wherein said agent is an agonist of SCD1 biological activity.
114. The process of claim 112 wherein said inhibitor does not substantially inhibit the biological activity in a human of a delta-5 desaturase, delta-6 desaturase or fatty acid synthetase
- 20 115. The process of claim 111 further comprising the step of assaying said therapeutic agent to further select compounds which do not substantially inhibit in a human the activity of delta-5 desaturase, delta-6 desaturase or fatty acid synthetase.
- 25 116. The process of claim 111 wherein said SCD1 biological activity is measured by an assay selected from among:
- 30 a) SCD1 polypeptide binding affinity;
- b) SCD1 desaturase activity in microsomes;
- c) SCD1 desaturase activity in a whole cell assay

- d) quantification of SCD1 gene expression level; and
- e) quantification of SCD1 protein level.

117. The process of claim 116 employing the cell of claim 9.

5

118. The process of claim 117 wherein the identified compound is further selected from among those compounds that do not substantially inhibit in humans the biological activity of delta-5 desaturase, delta-6 desaturase or fatty acid synthetase.

10

119. The process of claim 116 employing SCD1 nucleic acid of claim 14 and/or SCD1 polypeptide of claim 17 for use in identifying compounds useful for treatment of a disorder or condition relating to serum levels of triglyceride or VLDL.

15

[0080] Previous work not using human subjects has shown that aberrant SCD biological activity in those organisms (but not specifying which isoform of SCD was responsible) may be implicated in various skin diseases, as well as such diverse maladies as cancer and multiple sclerosis, non-insulin-dependent diabetes mellitus, hypertension, neurological diseases, skin diseases, eye diseases, immune disorders, and cancer. Modulators discovered using the processes of the present invention would thereby also find use in treating those diseases and disorders in human subjects.

[0171] Physiological benefits of an increase or decrease in the activity or expression of hSCD1 include, but are not limited to, decreased plasma triglycerides and/or increased plasma HDL leading to cardioprotective benefit, therapeutic benefit in Type II diabetes, weight loss, improved gland secretions, and decreased chance of malignancy. Thus, the determination of the ability of agents to modulate such activity or expression affords an opportunity to discover useful therapeutic agents producing such effects.

[0172] In addition, variations in hSCD1 gene expression, function, stability, catalytic activity and other characteristics may be due to allelic variations in the polynucleotide sequences encoding such enzymes. The processes disclosed according to the present invention may likewise be used to determine such genomic effects on expression of hSCD1. Using the processes of the present invention, such variations may be determined at the level of DNA polymorphism within the hSCD1 gene and/or promoter sequences. Such effects lead to the elucidation of associations between such polymorphisms and predisposition to cancer, neurological disease, skin disease, obesity, diabetes, immune function and lipid metabolism through both population and family-based genetic analysis.

[213] In other aspects, the present invention contemplates agents wherein said agent is useful in treating, preventing and/or diagnosing a disease or condition which is identified as being SCD1 related according to this invention. Specific embodiments are directed to situations wherein the disease or condition includes, but is not limited to, serum levels of triglyceride, VLDL, HDL, LDL, total cholesterol, reverse cholesterol transport or production of secretions from mucous membranes, monounsaturated fatty acids, wax esters, and the like, cholesterol disorders, lipidemias, cardiovascular disease, diabetes, obesity, baldness, skin diseases, cancer and multiple sclerosis, especially where the disease is a cardiovascular disease or a skin disease or where the condition is baldness. In a preferred embodiment, such agents will increase HDL levels in a patient and/or decrease triglyceride levels in a patient. Either or both effects are directly associated with reduced risk of cardiovascular disease and coronary artery disease.

[0229] In an additional aspect, the present invention also relates to a process for diagnosing a disease or condition in a patient, commonly a human being, suspected of being afflicted therewith, or at risk of becoming afflicted therewith, comprising obtaining a tissue sample from said patient and determining the level of activity of hSCD1 in the cells of said tissue sample and comparing said activity to that of an equal amount of the corresponding tissue from a patient not suspected of being afflicted with, or at risk of becoming afflicted with, said disease or condition. In specific embodiments thereof, said disease or condition includes, but is not limited to, cholesterol disorders, lipidemias, cardiovascular disease, diabetes, obesity, baldness, skin



diseases, cancer and multiple sclerosis, especially wherein said disease is a cardiovascular disease or a skin disease or said condition is baldness.